

LISTING OF CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims

1. **(Currently Amended)** A reaction mixture for primer-based amplification and detection of a target nucleic acid, the reaction mixture comprising:
each conventional nucleotide dATP, dCTP, and dGTP, and a dTTP in combination of
with dUTP and as a replacement for a portion of the dTTP in an amount generally equivalent to
the concentrations of dATP, dCTP and dGTP, wherein said dUTP is at a concentration of about
10% to about 100% of the concentration~~replaces from about 10 to about 50%~~ of said dTTP in
said ~~combination~~reaction mixture; and at least one of a fluorescent probe, beacon or intercalating
dye;

wherein the inclusion of dUTP reduces the formation of primer aggregates during the
amplification reaction in comparison with an amplification reaction employing only conventional
nucleotides; and

wherein said reaction mixture lacks a uracil degradation enzyme.

2. **(Currently Amended)** The reaction mixture according to claim 1 [[or 31]],
wherein the dUTP replaces from about 10 to about 30% of the dTTP in said reaction mixture.

3. **(Currently Amended)** The reaction mixture according to claim 1 [[or 31]],
wherein the dUTP replaces from about 20 to about 40% of the dTTP in said reaction mixture.

4. **(Currently Amended)** The reaction mixture according to claim 1 [[or 31]],
further comprising at least one additional unconventional nucleotide, wherein the combined
concentration of said dUTP and said at least one unconventional nucleotide does not exceed 75%
of any one conventional nucleotide in said reaction mixture.

5. **(Currently Amended)** The reaction mixture according to claim 1 [[or 31]],
wherein said reaction mixture comprises a primer pair and wherein each member of the primer
pair has at least one or more uracil bases incorporated therein.

6. **(Original)** The reaction mixture according to claim 5, wherein each member of
the primer pair has all of its thymidine bases replaced with uracil bases.

7. **(Currently Amended)** The reaction mixture according to claim 1 [[or 31]],
wherein the dUTP does not exceed a final amplification reaction concentration of about 300 μ M.

8. **(Currently Amended)** The reaction mixture according to claim 1 [[or 31]], wherein the dUTP does not exceed a final amplification reaction concentration of about 100 μ M.

9. **(Currently Amended)** The reaction mixture according to claim 1 [[or 31]], further comprising at least one polymerase enzyme.

10. **(Currently Amended)** The reaction mixture according to claim 1 [[or 31]], further comprising a buffer system.

11. **(Currently Amended)** A method for reducing primer aggregation during amplification and detection of target nucleic acid, the method comprising:

combining a target nucleic acid with a reaction mixture comprising each conventional nucleotide dATP, dCTP, and dGTP and a dTTP in combination of dTTP with dUTP in an amount generally equivalent to the concentrations of dATP, dCTP and dGTP as a replacement for a portion of the dTTP; wherein said dUTP is at a concentration of about 10% to about 100% of the concentration replaces from about 10 to about 50% of said dTTP in said combination~~reaction mixture~~;

amplifying the target nucleic acid to produce amplicons; and

detecting the amplicons so produced;

wherein the level of primer aggregate formed during the amplification step is reduced as compared to amplifying the target nucleic acid using a dNTP mix having only conventional nucleotides, wherein said method lacks an enzyme degradation step to degrade uracil-containing amplicons, and wherein said nucleic acid is DNA.

12. **(Canceled)**

13. **(Previously presented)** The method according to claim 11, wherein the reaction mixture further comprises sorbitol or mannitol.

14. **(Original)** The method according to claim 13, wherein the target nucleic acid has secondary structure.

15-18. **(Canceled)**

19. **(Previously presented)** The method according to claim 13, wherein the reaction mixture comprises 100 to 500 mM sorbitol or 100 to 300 mM mannitol .

20. **(Currently Amended)** The reaction mixture according to claim 1[[or 31]], wherein the reaction mixture further comprises sorbitol or mannitol.

21. **(Previously presented)** The reaction mixture according to claim 20, wherein the reaction mixture comprises 100 to 500 mM sorbitol or 100 to 200 mM mannitol.
- 22.-23. **(Cancelled)**
24. **(Currently Amended)** The method according to claim 11[[or 12]], wherein the dUTP replaces from about 10 to about 30% of the dTTP in said reaction mixture.
25. **(Currently Amended)** The method according to claim 11[[or 12]], wherein the dUTP replaces from about 20 to about 40% of the dTTP in said reaction mixture.
26. **(Currently Amended)** The method according to claim 11[[or 12]], further comprising at least one additional unconventional nucleotide, wherein the combined concentration of said dUTP and said at least one unconventional nucleotide does not exceed 75% of any one conventional nucleotide in said reaction mixture.
27. **(Currently Amended)** The method according to claim 11[[or 12]], wherein the dUTP does not exceed a final amplification reaction concentration of about 300 μ M.
28. **(Currently Amended)** The method according to claim 11[[or 12]], wherein the dUTP does not exceed a final amplification concentration of about 100 μ M.
29. **(Currently Amended)** The method according to claim 11[[or 12]], further comprising at least one polymerase enzyme.
30. **(Currently Amended)** The method according to claim 11[[or 12]], further comprising a buffer system.
31. **(Canceled)**
32. **(New)** The method according to claim 11, wherein said amplifying step comprises heating said target nucleic acid to a temperature greater than 60° C.